23. (Amended) A method for treating a human with sickle cell anemia comprising administering to the human an effective amount of a preparation comprising SNO-Hb(FeII)O_2.

Please add the following claims.

30. A method for regulating delivery of oxygen and NO, in various redox forms, in a mammal, comprising administering to the mammal an effective amount of a preparation comprising a low molecular weight thiol or nitrosothiol and S-nitrosohemoglobin.

31. A composition comprising SNO-Hb[FeII]O_2.

32. A composition comprising SNO-Hb[FeII].

REMARKS

Claims 15, 16 and 18-23 have been amended. Claims 30-32 have been added.

Support for Claim 30 can be found in the specification on page 10, line 30 to page 11, line 19, on page 12, lines 9-15, on page 13, line 7 to page 14, line 3, and in the original Claim 15, for instance.

Support for Claim 31 can be found, for example, on page 11, line 29 to page 12, line 2.

Support for Claim 32 can be found, for example, on page 12, lines 3-8.

Rejection of Claims 15-27 Under 35 U.S.C. § 112, Second Paragraph

Claims 15-27 have been rejected under 35 U.S.C. § 112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." Claims 15-27 are said to be "indefinite as to the mode of administration and the administrative amounts." The Examiner has suggested that the subject

claims be amended to recite "an effective amount." Claims 15, 16 and 18-23 have been so amended, thereby obviating the rejection of Claims 15-27.

Rejection of Claim 15 Under 35 U.S.C. § 102(b), Or, in the Alternative, Under 35 U.S.C. § 103(a)

Claim 15 has been rejected under 35 U.S.C. § 102(b) as being anticipated by Stamler *et al.*, WO 93/09806, or, in the alternative, under 35 U.S.C. § 103(a) as being obvious over Stamler *et al.*, WO 93/09806.

WO 93/09806 (Stamler *et al.*) describes the *in vitro* synthesis of various S-nitrosoproteins and nitrosylated amino acids, and demonstrates vasodilatory effects and anti-platelet effects of some of the S-nitrosoproteins.

As stated in the Declaration of Jonathan S. Stamler and the Declaration of Joseph Bonaventura (executed Declarations mailed to the United States Patent and Trademark Office on February 27, 1998 and March 12, 1998), at the time the subject application was filed, it was not known that S-nitrosohemoglobin could be made *in vitro*, or could exist under physiological conditions. Thus, a therapy using a combination of a low molecular weight thiol or nitrosothiol and S-nitrosohemoglobin could not have been known and could not have been obvious.

No therapy using a combination of low molecular weight thiol or nitrosothiol and hemoglobin is taught or suggested in WO 93/09806. Nowhere is the administration of hemoglobin alone or in combination with anything else taught or suggested.

On page 21, lines 17-18, WO 93/09806 suggests that the administration of "S-nitrosoproteins would deliver NO, and thus nitrosylate hemoglobin or myoglobin in order to increase oxygen binding." It might be inferred from this that the administration of S-nitrosoproteins alone might convert hemoglobin already present to S-nitrosohemoglobin. However, WO 93/09806 does not show that S-nitrosohemoglobin can even be made, *in vitro* or *in vivo*. There is no suggestion in this that hemoglobin be administered with anything else. As for the suggestion that nitrosylated hemoglobin would increase oxygen binding, no data are presented in WO 93/09806 to support this. On the contrary, the prior art teaches that hemoglobin acts as a scavenger of NO·. See Lancaster , J.R. et al., *Proc. Natl. Acad. Sci., USA 91*:8137-8141

(1994), for example, Figure 3A and 3B, page 8139 (reference cited as AV3). Low molecular weight nitrosothiols can act as donors of NO. The reaction of NO with hemoglobin produces NO_3^- and methemoglobin, which is not capable of binding oxygen. See also Furchgott, R.F., "Bioassays with Isolated Vascular Tissue for Endothelium-derived Relaxing Factor, Nitric Oxide and Nitric Oxide Donors," pages 567-581 In *Methods in Nitric Oxide Research,* Feelisch, M. and J.S. Stamler, eds., John Wiley & Sons, Chichester (1996), especially the section entitled "Hemoglobin (Hb)" on page 578 (reference provided herewith as Exhibit 1).

On page 23, lines 11-15, WO 93/09806 states: "An additional embodiment of the invention involves the *in vivo* nitrosylation of protein thiols, by administration of a nitrosylating agent as a pharmaceutical composition. *In vivo* nitrosylation provides a means for achieving any of the physiological effects discussed above, or for regulation of additional protein functions." From this it might be inferred that one could use a "nitrosylating agent" to produce S-nitrosohemoglobin *in vivo*. However, there was no evidence at the time the subject application was filed that S-nitrosohemoglobin could exist.

On page 2, lines 2-13 of WO 93/09806, the possible physiological role of low molecular weight thiols is discussed. However, their administration in a method of therapy is not discussed or implied, either alone or in combination with anything else.

The prior art teaches against the combination of hemoglobin with low molecular weight nitrosothiol. See, for example, Feelisch, M. *et al., Nature 368*:62-65 (1994), wherein it is described that hemoglobin inhibits the vasorelaxant activity of low molecular weight nitrosothiols in a cascade superfusion bioassay system using three precontracted de-endothielized strips of rabbit aorta (Table 1; reference provided herewith as Exhibit 2).

Rejection of Claim 15 Under 35 U.S.C. § 103(a)

Claim 15 has been rejected under 35 U.S.C. § 103(a) as being unpatentable over Stamler et al. (WO 93/09806) in view of Feola *et al.*, US 5,439,882, Klatz *et al.*, US 5,385,314 and Hunter, US 5,152,979.

The teachings of Stamler *et al.* (WO 93/09806) have been described above. As discussed above, the teachings of WO 93/09806 are limited, according to the statements in the Declaration of Jonathan S. Stamler and the Declaration of Joseph Bonaventura.

Feola *et al.* (US 5,439,882) describe cross-linked mammalian hemoglobin, a method of making the same, and a method of using the same as a blood substitute. Reduced glutathione is used in this reference to stop the cross-linking of hemoglobin when using o-adenosine as a cross-linking agent; glutathione becomes part of the cross-linked hemoglobin compound. See column 13, lines 2-6 and lines 27-30. The reported function of glutathione is as an "oxidant trap" (column 13, lines 7-14). Feola *et al.* do not suggest any role of glutathione in carrying out the biological functions of NO. Feola *et al.* do not teach or suggest any method of therapy by the administration of both hemoglobin and a low molecular weight thiol or nitrosothiol.

Klatz *et al.* (U.S. 5,395,314) describe an apparatus and a method to preserve organs in a cadaver or in a brain-dead patient before the organs can be removed for transplantation. The method employs a solution containing perfluorocarbons, which are to act as a blood substitute and transport oxygen in a manner similar to oxygen transport by hemoglobin. The solution may also contain antioxidants as free radical scavengers. Klatz *et al.* do not teach or suggest any use of hemoglobin with either a low molecular weight thiol or nitrosothiol.

Hunter (U.S. 5,152,979) describes a method for treating vascular obstructions, including those which may be caused by infection, sickle cell crisis, malaria and myocardial infarction. The method is to administer to a patient a surface active copolymer of a certain class of hydrophobes to reduce surface tension and friction in blood vessels, thereby reducing the incidence of thrombosis. Hunter does not teach or suggest the use of any form of hemoglobin, either alone or in combination with either a low molecular weight thiol or nitrosothiol.

In any attempt to combine the teachings of the cited references, Klatz *et al.* and Hunter. can be eliminated, because they neither teach nor suggest the administration of hemoglobin or of a low molecular weight thiol or nitrosothiol. Combining the teachings of WO 93/09806 with Feola *et al.*, one might find that WO 93/09806 could suggest the administration of S-nitrosoproteins or low molecular weight thiols alone for vasodilation or anti-platelet therapy. However, no reason is given in WO 93/09806 or the other cited references why one might want

to combine these agents with anything else, or with hemoglobin in particular. Feola *et al.* might suggest the use of glutathione as an integral part of a cross-linked preparation of hemoglobin, but does not teach or suggest any other separate use of glutathione, or of other low molecular weight thiols or nitrosothiols. No combination of the cited references can logically suggest the method of Claim 15.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (781) 861-6240.

Respectfully submitted,

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